Application Note

Automated buffer exchange during the protein characterization process

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Introduction

Biopharmaceutical drugs are currently the most successful group of drugs. Of these, therapeutic proteins and antibodies are the most popular. Due to the complexity of the molecules, in-depth characterization is very important. Quite often, a buffer exchange after the purification is necessary, in order to stabilize the protein and/or create conditions which are suitable for further processing.

scienova and Hamilton have partnered to automate scienova's Xpress Dialyzer plates on Hamilton's Microlab STAR platforms, in order to overcome the bottleneck in automating the entire workflow of therapeutic protein quality control.

- Easy to use and handle
- High recovery rate in all volume ranges
- Different cut offs available

Figure 1 | The Hamilton Microlab® STARlet

Automated workflow with scienova and Hamilton

Volume Recovery

During the entire process it is important not to lose too much protein in the buffer exchange chamber. The use of RC-regenerated cellulose membranes strongly reduces the binding of proteins to the membrane.

However, the protein loss during the pipetting process is also of importance. Therefore, different volumes of a test solution containing para-nitrophenol were filled in the cavities of different Xpress Dialyzer plates (Figure 2) without prewetting the membranes.

The cavities of the Xpress dialyzer plates were filled and emptied (from the top) with tips, as shown in Figure 3. The resulting amount of para-nitrophenol was quantified in an absorbance reader.

The recovery rates (even with low volumes) are always higher or equal to 75%; at higher volumes even above 90%, as shown in Figure 4.

Figure 2 | MD100 loaded into a 96 Deep Well plate

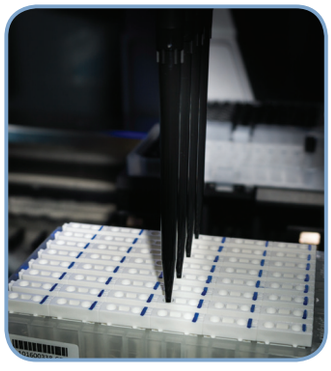

Figure 3 | Top filling and emptying of a MD1000 Mini Dialyzer

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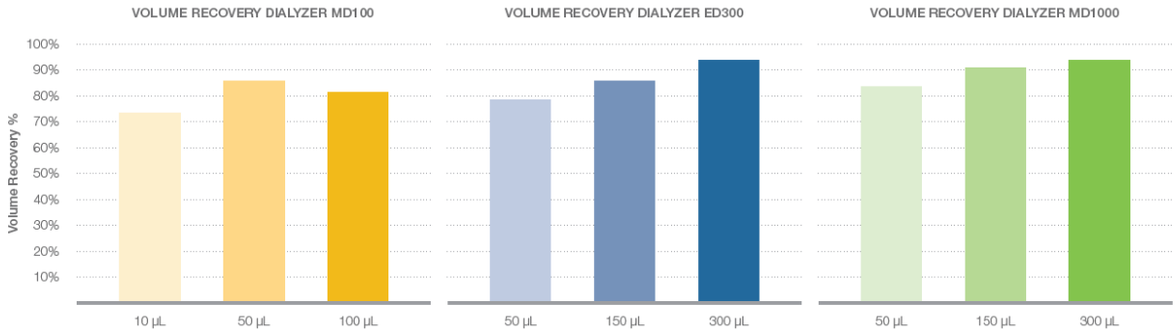

Figure 4 | Volume recovery rates for MD100, ED300 and MD1000 dialyzer plates

Equilibrium Dialysis

The time required to achieve a significant buffer exchange is important for the purification development strategy, in order to get the protein (as fast as possible) back into buffer conditions under which the protein is stable.

The robot filled the deep well plate with PBS buffer through the top inlet of the Xpress Dialyzer plates ED300, followed by transferring a BSA solution containing para-nitrophenol into the dialysis chamber as previously shown.

Samples were taken out of the deep well plate and the dialysis chamber, in order to observe the deviation of para-nitrophenol between both chambers. The equilibrium dialysis experiment was performed at room temperature with plates which have a different cut-off, in order to observe the influence of it on the time required. As shown in Figure 5, the equilibrium is reached after ~4h in all plate types; the first buffer exchange can already be achieved after ~2h, in order to optimize the entire buffer exchange rate.

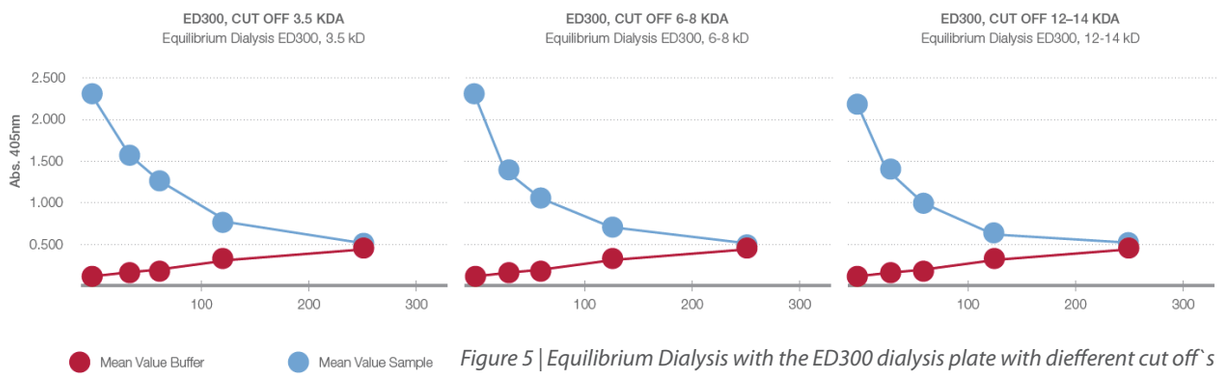

Figure 5 | Equilibrium Dialysis with the ED300 dialysis plate with diefferent cut off`s

Conclusion

The scienova Xpress Dialyzer plates are the ideal tool for an automated buffer exchange process during the quality control process of therapeutic proteins. The plates can be easily filled and emptied with Hamilton's Microlab STAR, even if a cooled environment is needed during the dialysis.

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